FOOD SECURITY IN NIGERIA: AGRICULTURAL DIVERSIFICATION AS A PANACEA

1st

International Conference of Agriculture and Agricultural Technology

{ICAAT 2019}

VENUE: **Federal University of Technology, Minna**
School of Agriculture and Agricultural Technology

DATE: **23rd to 26th April, 2019**

EDITED BY

Dr. C. E. Chinma
Mr. A. Ibrahim
Prof. J. N. Nmadu

- Prof. S. O. E. Sadiku
- Prof. R. S. Olaleye
- Prof. A. S. Gana
- Dr. M. A. Ojo

- Prof. A. Aremu
- Dr. A. J. Jirgi
- Dr. S. O. Olayimika
- Dr. B. A. Alimi

OCCURRENCE AND DISTRIBUTION OF PEPPER (*Capsicum* spp.) VIRUSES IN NIGER STATE, NIGERIA

Adama*, C. J., Salaudeen, M. T. and Bello, L. Y.

Department of Crop Production, School of Agriculture and Agricultural Technology,
Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria
*Corresponding Author's E-mail: cinwonj@gmail.com, GSM: 07065598936

ABSTRACT

Pepper is an important global food crop for the supply of vitamins and minerals for proper human growth. Virus diseases are responsible for huge losses in crop production and quality all over the world. Therefore, there is need for continuous survey in order to identify the virus types within a particular area which can be used by plant breeders to develop resistant cultivars. The objective of the study was to determine the incidence and distribution of pepper viruses in some Local Government Areas of Niger State. Surveys were carried out during the 2018 dry and wet seasons in selected Local Government Areas (Bida, Bosso, Mashegu, Mokwa and Wushishi) of Niger State, Nigeria. One hundred symptomatic leaf samples of pepper plants were collected randomly from 20 fields and were analyzed for viruses using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) with three polyclonial antibodies (PAbs) for Cucumber mosaic virus (CMV), Pepper venial mottle virus (PVMV) and Potato virus Y (PVY). Symptoms observed on fields included leaf chlorosis, mosaic, mottling, leaf and fruit deformation and stunting. All the tested samples reacted negatively to Potato virus Y (PVY) PAb. In all, 5 % of the samples collected from Bosso Local Government Area (LGA) reacted positively to CMV PAb. However, 65 % of the samples collected from Bida LGA tested positive for PVMV. This was closely followed by 25 % positive reaction to PVMV in Mashegu LGA. Moreover, 10 % positive reaction to PVMV was found in the samples collected at Bosso LGA. The study revealed the prevalence of PVMV and the presence of CMV in the study area. In order to prevent severe yield losses, pepper farmers should adhere to proper sanitation, use of clean seeds and rouging of infected plant stands. The inocula of these viruses can be used for breeding available CMV and PVMV resistant pepper cultivars.

KEYWORDS: Pepper viruses, DAS-ELISA, Niger State, CMV, PVMV

INTRODUCTION

Pepper (*Capsicum* spp.) originated from South and Central America and is a member of the Solanaceae family. It is one of the world's most popular vegetables which can be consumed fresh or processed and used mainly as a spice and condiment. In 2016, the world production of peppers was estimated at 546.3 million tonnes. Nigeria was the largest producer in Africa with about 67, 000 tonnes (FAO, 2016). Virus diseases annually reduce yield and quality of pepper. Symptoms of virus infection vary widely in expression and severity including mild mottle, mosaic, vein banding, ring spots, necrosis, leaf discoloration, deformation and blistering and severe stunting of the whole plant.

Studies have shown that about 40 viruses infect peppers (Kim *et al.*, 2009). The genus *Potyvirus*(family *Potyviridae*) containing about 200 species accounts for almost 25 % of known plant viruses. Species belonging to this genus can share many common properties. Arogundade*et al.* (2012) has reported high incidence of *Pepper veinal mottle virus* (PVMV) and *Cucumber mosaic virus* (CMV) in Nigeria. Other studies have also revealed the occurrence of *Potato virus Y* (PVY), *PotatovirusX* (PVX), *Pepper mild mottle virus* (PMMV), *Tobacco mosaic* virus (TMV), *Tobacco etch virus* (TEV) and *Tomato mosaic virus* (ToMV) (Arogundade*et al.*, 2015). *Cucumber mosaic vīrus* is a single-stranded RNA virus, tripartite and 29 mm in diameter. Virions contain 18 and 82 % nucleic acid and protein, respectively. Particles are found in all parts of the host specifically in the cytoplasm and inclusion bodies are present in infected cells (Roossinck, 2013). *Cucumber mosaic virus* has a wide host range and can cause complete crop loss. *Pepper veinal mottle virus* is transmitted by several species of aphids. It can also be transmitted mechanically. Leaves of infected plants show chlorotic vein banding, mottling mosaic and distortion with puckering. Losses could be as high as 90 %.

Similarly, *Potato virus Y* (PVY) also induces severe crop losses in pepper production. Symptoms of PVY disease include plant stunting, systemic vein-clearing, leaf mosaic or mottling, and dark green vein-banding

of the leaves. Necrosis in the veins and petioles often develop. This may be followed by stem necrosis and defoliation, death of the top bud and plant death. Affected fruit may be smaller, deformed, and with a mosaic pattern. *Potato* virus *Y* symptoms may be masked by symptoms of other viruses (AVRDC, 2004).

Identification of the viruses infecting pepper in Niger State would be useful for designing control measures. This can be achieved by using identified viruses infecting pepper for screening against the available locally adapted pepper accessions. Identification of high virus resistant and high yielding accessions would in turn lead to increased production and food security (Elvis *et al*., 2014). Therefore, the objective of the study was to determine the incidence and distribution of pepper viruses in some Local Government Areas of Niger State.

METHODOLOGY

Survey

Surveys were carried out to determine the occurrence and distribution of pepper viruses during the 2018 dry and wet season in selected Local Government Areas of Niger State (Bida, Bosso, Mashegu, Mokwa and Wushishi). A total of 100 leaf samples were collected from 20 pepper fields from infected plants showing symptoms such as chlorosis, mosaic, mottling, leaf and fruit deformation and stunting. Young leaf samples of infected plants were collected and stored in vial bottles containing self-indicating silica gels and non-absorbent cotton wool until analyzed. The coordinates of each farm were captured using the Geographical Positioning System (GPS) equipment (GPS- 4300; Ethrex Garmin GPS, Taiwan). Concise information including date and time of visit, farm size, cropping system, crops in neighbouring fields, fertilizer applications, insect pest and disease management strategies was recorded.

Virus Identification and Data Analysis

Leaf samples were tested for the presence of CMV, PVMV and PVY using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA). DAS-ELISA was performed in accordance with the manufacturer's instructions using CMV, PVMV and PVY specific polyclonal antisera purchased from Leibniz-institut, Deutsche Sammlung von Mikroorganismen und ZellkulturenBraunschweig, Germany.

For DAS-ELISA, the wells of polystyrene microplates (Corning, NY 14831, USA) were coated with 200 µL of the corresponding antiserum diluted to 1000-fold (1:1000) in coating buffer (0.05 M sodium carbonate, pH 9.6) and incubated at 37 °C for 2 hours. Incubated plates were washed three times with PBS-Tween (phosphate-buffered saline-Tween containing PBS and 0.5 ml Tween 20 per litre). Plates were blotted by tapping upside down on tissue paper. Samples were extracted (1:20 w/v) in extraction buffer [containing PBST and 2 % PVP (e.g. Serva PVP – 15 polyvinyl pyrrolidone). Two hundred µL aliquots of each sample were added to duplicate wells, covered and incubated overnight at 4 °C. Extraction buffers were used as negative controls. The incubated plates were washed three times, followed by addition of 200 µL alkaline phosphatase (AP) conjugated IgG at 1:1,000 dilution in conjugate buffer (phosphate-buffered saline, pH 7.4, containing 0.05 % Tween 20 and 0.2 % egg albumin). The plates were covered and again incubated at 37 °C for 2 hours. Thereafter, the plates were washed three times as described above and blotted on tissue paper. The alkaline phosphatase substrate tablets (Sigma-Aldrich, St. Louis, MO, USA) were prepared in the substrate buffer (9.7 % di-ethanolamine, pH 9.8) to a final concentration of 1 mg mL^{-1}. One hundred microliters of *p*-nitrophenyl phosphate substrate was added to each well and the plates were incubated for 60 minutes at 37 °C. Absorbance values were read at 405 nm using a microplate reader (iMark Microplate Absorbance Reader (Bio-Rad), Germany). Samples were considered to be positive when the mean of the absorbance values at 405 nm (A_{405}) was twice the negative controls. Disease incidences were calculated as percentage of total number of infected samples that tested positive for the viruses.

RESULTS AND DISCUSSION

Pepper in the surveyed areas was grown on not less than one hectare of land for sale and also for family consumption. Farmers cultivated pepper twice a year both during the dry and wet seasons. Pepper cultivation during the dry season is made possible with irrigation and proximity to source of water usually rivers. Pepper was grown in mixtures with other principal food crops such as maize, cassava, cowpea, millet, rice, okra and groundnut. The farmers interviewed stated that they usually buy seeds from the market or from previous harvest. Similarly, weeds were controlled manually and pesticides were used to control insect pests and diseases. Inorganic fertilizers such as NPK and urea were the main source for soil improvement. The presence of some diseased conditions was attributed to the late arrival of rain. The farmers were willing to source seeds and other agricultural inputs from reliable sources like the Research institutes, Ministries of Agriculture and Agricultural Development Projects (ADPs).

The symptoms observed on the pepper fields were mosaic, yellowing and leaf mottling. About 5 % of the samples exhibited strong positive reaction to CMV antibody (Fig. 1 and Table 1). The incidence of CMV was observed at Dokumgba in Bida LGA. *Cucumber mosaic virus* was not detected in Bosso, Wushishi, Mokwa and Mashegu LGA. Ten percent of the indexed samples collected in Bosso LGA showed strong positive reaction to PVMV antibody (Fig. 1 and Table 2). The samples that tested positive for PVMV were collected at Angwan-Gwari and Chanchaga. Furthermore, 20 % of the samples collected from Wushishi LGA exhibited strong positive reaction to PVMV antibody. The PVMV incidence in Wushishi was observed at Bogi and Mailema with higher incidence at Mailema. In Mokwa LGA, 15 % PVMV disease incidence was observed. Incidence of PVMV was highest at Muwo (5 %); this was closely followed by Mokwa township (5 %) and Bokani (5 %). Additionally, 25 % of the samples were positive for PVMV antibody (Table 2) in Mashegu LGA. The positive samples were collected from Makera (5 %) and Manigi (20 %). In Bida LGA, 65 % of the samples tested positive for PVMV PAbs. The incidence of PVMV was wide spread across all the farms visited in Bida LGA at Dokumgba (60 %), and Kuchi (5 %). All the samples reacted negatively to PVY antibody (Table 3).

The serological method used revealed the occurrence of two viruses in pepper farms across the surveyed areas as single infections. *Pepper veinal mottle virus* was the most occurring virus followed by CMV. The higher incidence of PVMV observed in Bida LGA implied that it was a hotspot of the virus. *Pepper veinal mottle virus* (PVMV) has been previously reported as one of the most prevalent viruses infecting pepper in south west Nigeria (Fajinmi, 2010). The high occurrence of PVMV could be attributed to its ability to survive in weed hosts for relatively long period of time (Agrios, 2005) and other vegetables. This confirms the findings of Alegbejo (2015) who reported that the nearness of pepper plants to certain important weed hosts also has contributed greatly to the spread of virus diseases of pepper. The wide spread of PVMV within crops could .occur due to mechanical transmission by farmers ranging from contaminated hands, clothing, and tools during routine farm operations such as transplanting, pruning, grafting, and other farm activities. Fajinmi*et al*. (2011) suggested that the incidence and severity on susceptible cultivars can be reduced or eliminated with the knowledge of the ecology and distribution of aphid vectors within a particular area.

CONCLUSION AND RECOMMENDATIONS

Adoption of early planting, integrated pest management, good field sanitation, weeding, and rouging of infected pepper stands will serve as other management techniques (Fajinmi*et al*., 2012). The inocula of the identified CMV and PVMV could be used for screening some available pepper cultivars for resistance and breeding purposes.

Table 1: Serological reactions of pepper leaves to *Cucumber mosaic virus* (CMV) antibody

Sample ID	CMV Antibody	Sample ID	CMV Antibody	Sample ID	CMV Antibody
BO 1.1	0.154	WU 7.5	0.161	MS 14.3	0.163
BO 1.2	0.135	WU 8.1	0.145	MS 14.4	0.155
BO 1.3	0.106	WU 8.2	0.146	MS 14.5	0.153
BO 1.4	0.157	WU 8.3	0.143	MS 15.1	0.142
BO 1.5	0.115	WU 8.4	0.156	MS 15.2	0.143
BO 2.1	0.157	WU 8.5	0.131	MS 15.3	0.141
BO 2.2	0.149	MO 9.1	0.129	MS 15.4	0.149
BO 2.3	0.114	MO 9.2	0.143	MS 15.5	0.181
BO 2.4	0.176	MO 9.3	0.142	MS 16.1	0.157
BO 2.5	0.148	MO 9.4	0.115	MS 16.2	0.171
BO 3.1	0.096	MO 9.5	0.097	MS 16.3	0.192
BO 3.2	0.124	MO 10.1	0.144	MS 16.4	0.166
BO 3.3	0.125	MO 10.2	0.111	MS 16.5	0.158
BO 3.4	0.147	MO 10.3	0.217	BD 17.1	0.155
BO 3.5	0.138	MO 10.4	0.176	BD 17.2	0.170
BO 4.1	0.157	MO 10.5	0.138	BD 17.3	0.172
BO 4.2	0.132	MO 11.1	0.185	BD 17.4	0.347*
BO 4.3	0.153	MO 11.2	0.155	BD 17.5	0.171
BO 4.4	0.168	MO 11.3	0.157	BD 18.1	0.161
BO 4.5	0.149	MO 11.4	0.148	BD 18.2	0.150
WU 5.1	0.115	MO 11.5	0.152	BD 18.3	0.192
WU 5.2	0.132	MO 12.1	0.175	BD 18.4	0.168
WU 5.3	0.132	MO 12.2	0.17	BD 18.5	0.170
WU 5.4	0.134	MO 12.3	0.191	BD 19.1	0.172
WU 5.5	0.154	MO 12.4	0.170	BD 19.2	0.174
WU 6.1	0.154	MO 12.5	0.215	BD 19.3	0.146
WU 6.2	0.161	MS 13.1	0.168	BD 19.4	0.153
WU 6.3	0.151	MS 13.2	0.132	BD 19.5	0.163
WU 6.4	0.128	MS 13.3	0.148	BD 20.1	0.170
WU 6.5	0.140	MS 13.4	0.152	BD 20.2	0.143
WU 7.1	0.151	MS 13.5	0.181	BD 20.3	0.150
WU 7.2	0.156	MS 14.1	0.157	BD 20.4	0.184
WU 7.3	0.158	MS 14.2	0.157	BD 20.5	0.137
WU 7.4	0.148				

Positive control = 0.272; Buffer control = 0.136; *Positive reaction;
BO = Bosso; WU = Wushishi; MO = Mokwa; MS = Mashegu; BD = Bida

Table 2:Serological reactions of pepper leaves to *Pepper veinal mottle virus* (PVMV) antibody

Sample ID	PVMV Antibody	Sample ID	PVMV Antibody	Sample ID	PVMV Antibody
BO 1.1	0.250	WU 7.5	0.192	MS 14.4	0.128
BO 1.2	0.164	WU 8.1	0.184	MS 14.5	0.118
BO 1.3	0.202	WU 8.2	0.143	MS 15.1	0.093
BO 1.4	0.246	WU 8.3	0.155	MS 15.2	0.163
BO 1.5	0.168	WU 8.4	0.114	MS 15.3	0.120
BO 2.1	0.216	WU 8.5	0.104	MS 15.4	0.125
BO 2.2	0.187	MO 9.1	0.076	MS 15.5	0.137
BO 2.3	0.229	MO 9.2	0.081	MS 16.1	0.155
BO 2.4	0.297*	MO 9.3	0.086	MS 16.2	0.706*
BO 2.5	0.173	MO 9.4	0.117	MS 16.3	0.813*
BO 3.1	0.208	MO 9.5	0.144	MS 16.4	0.300*
BO 3.2	0.152	MO 10.1	0.130	MS 16.5	0.576*
BO 3.3	0.107	MO 10.2	0.124	BD 17.1	0.467*
BO 3.4	0.100	MO 10.3	0.353*	BD 17.2	0.633*
BO 3.5	0.143	MO 10.4	0.089	BD 17.3	0.782*
BO 4.1	0.196	MO 10.5	0.124	BD 17.4	0.185
BO 4.2	0.226	MO 11.1	0.140	BD 17.5	0.785*
BO 4.3	0.162	MO 11.2	0.042	BD 18.1	0.504*
BO 4.4	0.305*	MO 11.3	0.086	BD 18.2	0.156
BO 4.5	0.243	MO 11.4	1.086**	BD 18.3	0.322*
WU 5.1	0.345*	MO 11.5	0.135	BD 18.4	0.211*
WU 5.2	0.201	MO 12.1	0.107	BD 18.5	0.471*
WU 5.3	0.102	MO 12.2	0.117	BD 19.1	0.446*
WU 5.4	0.317*	MO 12.3	0.096	BD 19.2	0.731*
WU 5.5	0.160	MO 12.4	0.441*	BD 19.3	0.359*
WU 6.1	0.171	MO 12.5	0.133	BD 19.4	0.440*
WU 6.2	1.053**	MS 13.1	0.453*	BD 19.5	0.151
WU 6.3	0.210	MS 13.2	0.111	BD 20.1	0.157
WU 6.4	0.085	MS 13.3	0.148	BD 20.2	0.177
WU 6.5	0.169	MS 13.4	0.128	BD 20.3	0.142
WU 7.1	0.538*	MS 13.5	0.160	BD 20.4	0.445*
WU 7.2	0.213	MS 14.1	0.130	BD 20.5	0.110
WU 7.3	0.222	MS 14.2	0.140		
WU 7.4	0.134	MS 14.3	0.119		

Positive control = 0.270; Buffer control = 0.135; *Positive reaction;
BO = Bosso; WU = Wushishi; MO = Mokwa; MS = Mashegu; BD = Bida

Table 3: Serological reactions of pepper leaves to *Potato virus Y* (PVY) antibody

Sample ID	PVY Antibody	Sample ID	PVY Antibody	Sample ID	PVY Antibody
BO 1.1	0.141	WU 7.5	0.140	MS 14.4	0.144
BO 1.2	0.126	WU 8.1	0.133	MS 14.5	0.145
BO 1.3	0.139	WU 8.2	0.128	MS 15.1	0.139
BO 1.4	0.134	WU 8.3	0.112	MS 15.2	0.126
BO 1.5	0.107	WU 8.4	0.101	MS 15.3	0.130
BO 2.1	0.130	WU 8.5	0.085	MS 15.4	0.145
BO 2.2	0.111	MO 9.1	0.111	MS 15.5	0.166
BO 2.3	0.150	MO 9.2	0.102	MS 16.1	0.166
BO 2.4	0.166	MO 9.3	0.101	MS 16.2	0.158
BO 2.5	0.167	MO 9.4	0.121	MS 16.3	0.153
BO 3.1	0.101	MO 9.5	0.117	MS 16.4	0.139
BO 3.2	0.107	MO 10.1	0.139	MS 16.5	0.142
BO 3.3	0.091	MO 10.2	0.116	BD 17.1	0.150
BO 3.4	0.112	MO 10.3	0.122	BD 17.2	0.137
BO 3.5	0.121	MO 10.4	0.113	BD 17.3	0.144
BO 4.1	0.105	MO 10.5	0.124	BD 17.4	0.153
BO 4.2	0.116	MO 11.1	0.144	BD 17.5	0.160
BO 4.3	0.105	MO 11.2	0.153	BD 18.1	0.149
BO 4.4	0.134	MO 11.3	0.143	BD 18.2	0.174
BO 4.5	0.131	MO 11.4	0.157	BD 18.3	0.180
WU 5.1	0.116	MO 11.5	0.171	BD 18.4	0.142
WU 5.2	0.131	MO 12.1	0.156	BD 18.5	0.160
WU 5.3	0.124	MO 12.2	0.174	BD 19.1	0.141
WU 5.4	0.126	MO 12.3	0.207	BD 19.2	0.150
WU 5.5	0.156	MO 12.4	0.205	BD 19.3	0.142
WU 6.1	0.147	MO 12.5	0.187	BD 19.4	0.152
WU 6.2	0.145	MS 13.1	0.163	BD 19.5	0.152
WU 6.3	0.135	MS 13.2	0.130	BD 20.1	0.214
WU 6.4	0.104	MS 13.3	0.149	BD 20.2	0.200
WU 6.5	0.135	MS 13.4	0.140	BD 20.3	0.151
WU 7.1	0.122	MS 13.5	0.147	BD 20.4	0.223
WU 7.2	0.122	MS 14.1	0.153	BD 20.5	0.139
WU 7.3	0.131	MS 14.2	0.148		
WU 7.4	0.124	MS 14.3	0.144		

Positive control =0.266; Buffer control = 0.133; *Positive reaction;
BO = Bosso; WU = Wushishi; MO = Mokwa; MS = Mashegu; BD = Bida

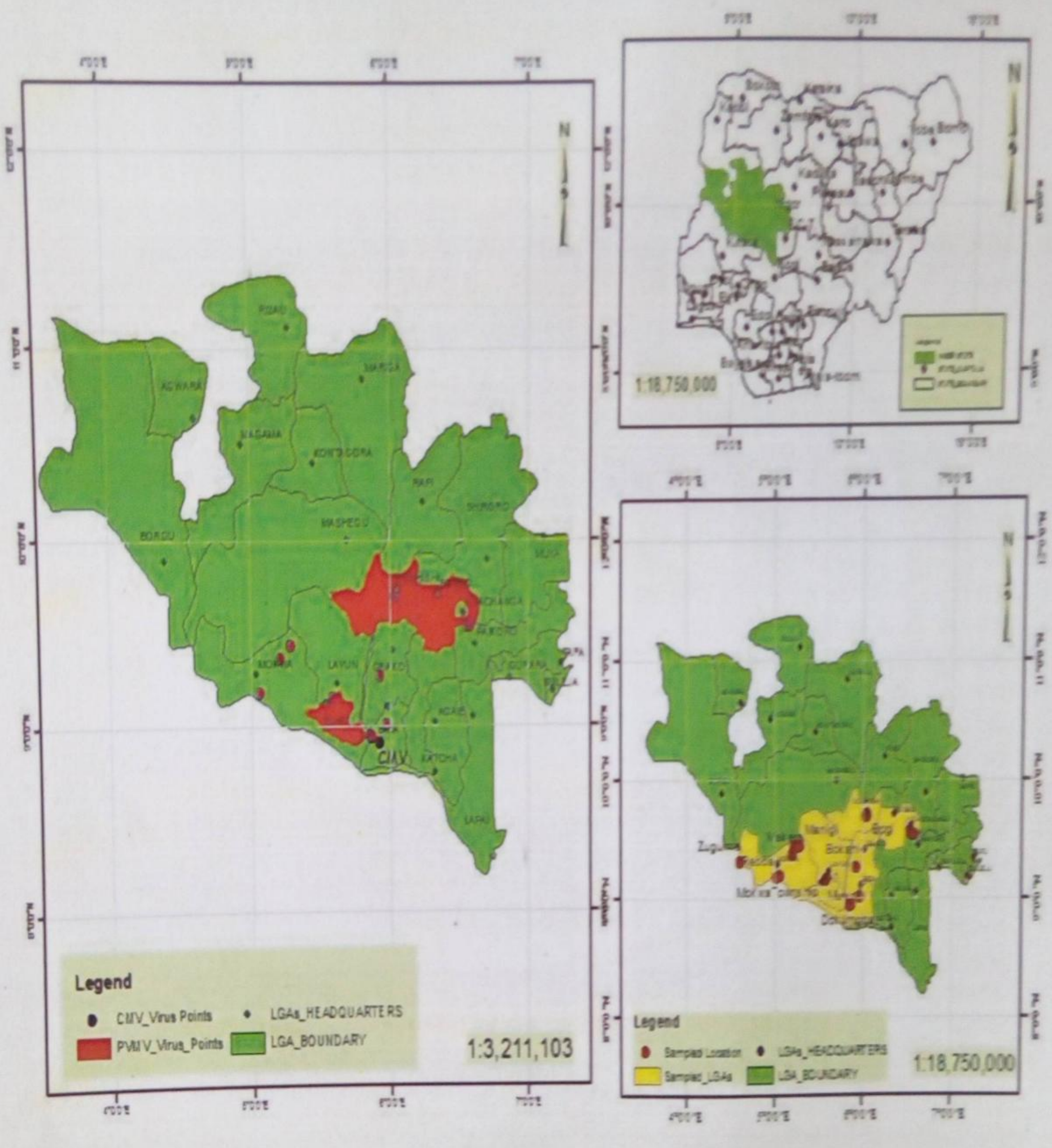

Fig. 1: Maps showing sampling points and distribution of *Cucumber mosaic virus* (CMV), *Pepper veinal mottle virus* (PVMV) and *Potato virus Y* (PVY) in Niger State, Nigeria

REFERENCES

Agrios G. N. (2005). Plant Pathology. Fifth Edition. Elsevier Academic Press. 30 Corporate Drive, Suite 400, Burlington, MA 01803, USA 525 B Street, Suite 1900, San Diego, California 92101-4495, USA 84 Theobald's Road, London WC1X 8RR, UK.

Alegbejo, M. D. (2015). Virus and virus-like diseases of crops in Nigeria. Zaria, Nigeria, Ahmadu Bello University Press, 12, 89-91.

Arogundade, O., Balogun, O. S. and Kareem, K. T. (2012). Occurrence and distribution of *Pepper veinal mottle virus* and *Cucumber mosaic virus* in pepper in Ibadan, Nigeria. *Virology Journal*, 9:79.

Arogundade, O., Balogun, O. S., Akinyemi, S. O. S. and Kumar, P. L. (2015). Surveys of virus diseases on pepper (*Capsicum spp.*) in South-west Nigeria. *African Journal of Biotechnology*, 14(48): 3198-3205.

Asian Vegetable Research and Development Center (AVRDC). (2004). Fact Sheet;*Peppermottlevirus*. Asian Vegetable Research and Development Center (AVRDC) Publication.

Elvis, A., Albert, A. and Appiah, B. (2014). Comparative Efficacy of Phytopesticides in themanagement of *Podagrica SPP.* And Mosaic Disease on Okra(*Abelmoschusesculentus* L.). *American Journal of Experimental Agriculture*, 4(8): 879-889.

Food and Agriculture Organization (FAO).(2016). Tomato production statistics. Available at http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor. Accessed on May 1, 2017.

Fajinmi, A. A. (2010). Agro-ecological incidence and severity of *Pepper veinal mottle virus*,genus *Potyvirus*, Family Potyviridae, on cultivated pepper (*Capsicum annuum* L.) in Nigeria. 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops. Julius-Kühn-Archiv, 427. pp. 314-322.

Fajinmi, A. A., Odebode, C. A. and Fajinmi, O. B. (2011). Incidence and distribution of aphid vectors of *Pepper veinal mottle virus* on bell pepper. *International Journal of Entomology*, 1(1): 1-12.

Fajinmi, A. A., Popoola, A. R. and Fajinmi, O. B. (2012). Incidence and epidemiology of *Pepper veinal mottle virus* (PVMV), a Potyvirus disease of pepper. *Archives of Phytopathology and Plant Protection*. DOI:10.1080/03235408.2012.694250.

Kim M., Suh, H., Cho, E. J. and Buratowski, S. (2009). Phosphorylation of the yeast Rpb1 C-terminal domain at series 2, 5, and 7. *Journal of Biological Chemistry*, 284(39): 264-271.

Roossinck, M. J (2013). Plant Virus Ecology. PLoS *Pathology*. 9(5): 134-139.